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Correlation of Liver Transaminases with Platelet count in Dengue patients from Tertiary Care Hospital in Western India

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ABSTRACT

Aim: Dengue is the arboviral febrile illness, which affects the vascular, muscular and hematological systems. Thrombocytopenia is one of the criteria used by WHO guidelines as a potential indicator of clinical severity of infection. Along with that hepatic dysfunction is a well-recognized feature of dengue infection characterized by hepatomegaly and increased serum transaminase levels. Aim of this study was To Assess the liver involvement in dengue infection during critical period of illness by correlating liver transaminase enzymes levels with platelet count in Tertiary care hospital in Western India.

Methods: 80 dengue patients were divided into Group A of 40 cases of dengue with platelet count less than 100000/cmm and Group B of 40 cases of dengue with platelet count more than 100000/cmm. 40 age and gender matched healthy controls were taken into Group C. Under all aseptic precautions, 2 ml of blood sample was collected from ante-cubital vein without occlusion in EDTA vacutainer and 1 ml in plain vacutainer. Separated serum was subjected for measurement of serum AST (Aspartate Transaminase) and ALT (Alanine Transaminase). Whole Blood from EDTA was used for Platelet Count Measurement.

Results: In our study we found levels of AST and ALT were higher in Group A and Group B compared to group C. Mean AST and ALT levels were much higher in group A as compared to group B. Also abnormality in AST levels is higher than ALT in both group A and group B. AST and ALT level > 1000 IU/L were recorded only in group A and not in group B which suggests that severe hepatic dysfunction in terms of rise in AST and ALT are mostly seen in patients with platelet count <1,00,000 /cmm during critical period of illness. When we correlated AST and ALT with platelet count in group A and group B, we found negative correlation between them in both groups. When we correlated platelet count with AST and ALT in all dengue patients irrespective of platelet count, we got not only negative correlation between them but also this correlation was statistically significant

Conclusion: Our study concludes that transaminase levels increases in almost all dengue patients. The rise in AST and ALT level increases with increase in dengue severity which is indicated by fall in platelet count as they are negatively correlated with each other. But this negative correlation cannot be used to differentiate dengue patients with platelet count <1,00,000 /cmm and > 1,00000/cmm. So liver damage is one of the common complications of dengue and AST as well as ALT levels should be used as biochemical markers in those patients to detect and monitor hepatic dysfunction.

Key Words: Dengue, AST, ALT, Platelet Count

INTRODUCTION

Dengue or break bone fever is an important and most widely distributed mosquito born viral infection causing febrile illness in tropical and subtropical region (1, 2). Dengue virus (DENV) is a single stranded RNA virus of flaviviridae family and consists of 4 serotypes as DEN- 1, DEN-2,DEN -3, DEN-4.(1) The virus is transmitted by the bite of Aedes aegypti mosquito (1,3). Manifestations of dengue virus infection vary from no symptoms, dengue fever (DF), dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS).(2,4,5) Around 100 million cases of DF and between 2, 50,000 to 5, 00,000 cases of DHF are annually reported to World Health Organization (WHO). (4) Classic dengue fever is characterized by fever, retro orbital headache and

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severe myalgia. (5) A more severe form of disease is DHF and dengue shock syndrome. (5,6) Abnormal hemostasis and plasma leakage are characteristic features of DHF which are clinically manifested as thrombocytopenia, spontaneous hemorrhage and haemoconcentration near the time of defervescence, typically after 5 days of fever (4,5,7). Thrombocytopenia is one of the criteria used by WHO guidelines as a potential indicator of clinical severity of infection. Also in the most recent WHO guidelines the definition generally describes rapid decline or a platelet count less than 1,50,000 per microliter of blood.(8) Dengue infection has effect on vascular, muscular and hematological system. (9)

WHO 1997 classification categorizes dengue infection as Dengue Fever (DF), Dengue Hemorrhagic Fever (DHF) and Dengue Shock Syndrome (DSS). (10)

DF classification requires fever and at least two of the following: headache, eye pain, myalgia, arthralgia, rash, bleeding, and leukopenia. (10)

Dengue hemorrhagic fever requires all of the following: fever, platelet count $\leq 100 \times 10^9$/liter, bleeding, and plasma leakage. (10)

Dengue shock syndrome is a case of DHF with either tachycardia or pulse pressure <20 mmHg or systolic blood pressure <90 mmHg. (10)

WHO 2009 guidelines categorize dengue infection into dengue without warning signs (WS), dengue with WS, or severe dengue. Dengue (WHO 2009) requires fever and two of the following: nausea, vomiting, rash, aches and pains, leucopenia, or any warning sign. Warning signs include abdominal pain or tenderness, persistent vomiting, clinical fluid accumulation, mucosal bleeding, lethargy or restlessness, hepatomegaly, or hematocrit rise (≥20%) with rapid drop in platelet count (<50,000/liter). (11)

Along with that hepatic dysfunction is a well recognized feature of dengue infection characterized by hepatomegaly and increase in serum transaminase levels.(2,9) This liver dysfunction could be direct viral effect on hepatocytes or a result of dysregulated host immune response against infection.(9) The inflammatory process resulting from paranchymatous lesion releases transaminases in blood.(12) Number of studies has reported that patients with dengue infection have elevated levels of liver transaminase enzymes. (2, 5, 9)

Taking into account the impact of dengue virus infection on liver, we decided to study involvement of liver in serologically diagnosed dengue patients by correlating the liver transaminase levels with platelet count during critical period (4-6 days of illness) of illness, as this period is more prone for complications of illness like DHF or DSS (4,7).

Platelet count is used as a parameter to correlate liver transaminase levels in dengue patients during critical period of illness with, as thrombocytopenia is one of the important criteria used by WHO to indicate severity of the dengue infection. (10, 11)

MATERIALS AND METHODS

This was a case control study which has been carried out at Bharati Vidyapeeth Deemed University Medical College and Hospital Pune. The study protocol was approved by the Institutional Ethics Committee. The present study was carried out on serologically diagnosed dengue patients of age more than 18 years irrespective of the gender. Diagnosis of dengue was confirmed by demonstration of anti DV IgM antibodies or by NS-1 antigen in patient's serum depending upon day of illness using Dengue day one solid phase immunochromatographic test kit. When result of the test was positive for IgM or NS1 Antigen, patients were considered currently to be infected by Dengue virus. Study population was comprised of 80 serologically diagnosed dengue patient which were subcategorized into 2 groups on the basis of platelet count as below:

Group A: 40 cases of dengue with platelet count less than 1,00,000/cmm

Group B: 40 cases of dengue with platelet count more than 1,00,000/cmm

While group C was comprised of 40 age and gender matched healthy controls.

Inclusion criteria:

Diagnosed cases of dengue with age above 18 years during 4-6 days of illness

Exclusion criteria:

Febrile illness other than dengue (DV IgM negative), patients with bleeding disorders or platelet dysfunction, patients of chronic liver disease, liver cirrhosis or alcoholic liver disease.

METHODS

Study details were explained to the subjects. Informed written consent was taken and blood samples were collected from cases during forth / fifth day of illness while random blood sample was taken from control group. Under all aseptic precautions, 2 ml of blood sample was collected from ante-cubital vein without occlusion in EDTA vacutainer and 1 ml in plain vacutainer.

Serum was separated after centrifugation at 2500 rpm for 10 minutes from plain vacutainer.

Separated serum was subjected for measurement of serum

AST and ALT. Whole Blood from EDTA vacutainer was used for measurement of platelet count. Commercially available kits of high sensitivity and specificity were used for analytical measurements. All the parameters measured are in the scope of accreditation by NABL. (Certificate No.MC-2181)

Sr. No.	Test	Method
1.	Dengue	Dengue day one solid phase immunochromatographic test kit.(13) (Antigen-Antibody reaction)
2.	Estimation of serum AST	Modification of the optimized standard method according to the recommendations of the IFCC on Fully Automated biochemistry analyzer Rx Imola.(14,15)
3.	Estimation of serum ALT	Modification of the optimized standard method according to the recommendations of the IFCC on Fully Automated biochemistry analyzer Rx Imola.(14,16)
4.	Platelet Count	Electrical impedence method in automated cell counter (17)

Quality Control

Commercially available control materials were used to check the quality of all assay procedures.

Statistical Analysis

Data analysis was done using the SPSS (Statistical Package for the Social Science) Version 11 for window. Pearson's correlation coefficient test was used to calculate the correlation between variables in each group.

A probability value of 0.05 was accepted as the level of statistical significance.

P-value less than 0.05 ($P < 0.05$) was considered to be statistically significant (S).

P-value more than 0.05 ($P > 0.05$) was considered to be statistically non-significant (NS).

RESULT

Mean age of patients in group A was **(31.8±13.7)** and Group B was **(33.57±15.17).** Mean age of Group C was **(34.73±9.95). (Table 1)**

Mean AST and ALT level was **(320 ± 513.38)** IU/L and **(164 ± 188.95)** IU/L respectively in group A. While median for AST and ALT were 220 IU/L and 120 IU/L respectively in group A.**(Table 2)**

Mean AST and ALT level was **(70±80.81)** IU/L and **(55±66.46)** IU/L respectively in group B. While median for AST and ALT were 38 IU/L and 32 IU/L respectively in group B.**(Table 3)**

Mean AST and ALT level was **(25.27±9.53)** IU/L and **(24.57±12.60)** IU/L respectively in group C. **(Table 4)**

Levels of serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) were higher seen in Group A and Group B which are of dengue patients as compared to group C. **(Table No. 2,3,4)**

When we correlated platelet count with AST and ALT in group A where platelet count is <1,00,000/cmm, we found negative correlation between AST (r =-0.26) and ALT (r =-0.29) with that of platelet count but that was not statistically significant (P value >0.05). **(Table 5) (Fig1, 2,).**

After correlating platelet count with AST and ALT in group B where platelet count is >1,00,000/cmm, we found negative correlation between ALT (r =-0.28) and platelet count which was not statistically significant (P value >0.05).

For AST also we found negative correlation (r =-0.42) with that of platelet count in group B and it was statistically significant (P value > 0.05). **(Table 6) (Fig3, 4).**

When we correlated platelet count with AST and ALT in all dengue patients irrespective of platelet count, we got not only negative correlation between AST(r =-0.36) and ALT (r =-0.39) with that of platelet count but also this correlation was statistically significant (P value <0.001). **(Table 7) (Fig 5,6)**

DISCUSSION

Dengue is an important arboviral disease whose manifestations varies from asymptomatic fever, to thrombocytopenia, and deranged hemostasis resulting in DHF or in severe cases circulatory compromise leading to life-threatening dengue shock syndrome (DSS). (1, 2, 3, 4, 5, 6)

Thrombocytopenia has always been one of the criteria used by WHO guidelines as a potential indicator of clinical severity of dengue infection. (8) Hepatic dysfunction is common in dengue infection which can be a direct effect of virus on liver cells or be a consequence of dysregulated host immune responses against the virus. (9, 23, 29)

Liver involvement in dengue can be biochemically detected with rise in liver transaminase enzyme levels. (2, 4, 9, 12)

In our study we tried to find out the correlation between platelet count which is an indicator of severity of dengue infection according to WHO 1997 as well as 2009 guidelines with liver transaminases. (10, 11)

Levels of serum aspartate aminotransferase (AST) and serum alanine aminotransferase (ALT) were higher seen in Group

A and Group B which are of dengue patients as compared to group C. **(Table No. 2,3,4)**

An elevation in serum AST level was seen in 95% (n=38) and in serum ALT level in 92.5% (n=37) of cases in group A. **(Table 2)**

Rise in serum AST level was seen in 40% (n=16) and that of in serum ALT level in 20% (n=8) of cases in group B. **(Table 3)**

Kuo et al also in their study of evaluation of 270 dengue patients found abnormal AST and ALT levels in 93.3 % and 82.2 % respectively. (18)

Also these findings are consistent with those of LA Villar-Centeno where they studied biochemical alterations in DHF and concluded that early alterations in biochemical markers can be used to predict DHF in dengue fever patients. (4)

In our study also only 5% (n=2) and 7.5% (n= 3) had normal (5-40 IU/L) AST and ALT levels respectively in group A with median platelet count of 48000/ cmm, while 60 % (n=24) and 80% (n=32) had normal levels (5-40 IU/L) of AST and ALT respectively in group B with median platelet count of 1, 78,500/cmm. **(Table 2, 3)**

So abnormality in AST levels is higher than ALT which goes with the finding of Wong and Shen. (19) This is also consistent with finding of study by Nguyen et al. which showed us that, AST values were reported to be frequently abnormal, reaching values higher than those of ALT, around 97.7% and 37.3% above normal levels, respectively. (20)

In our study, 32 (80%) cases had AST level more than 120 IU/L (> 3×ULN) and 24 (60 %) cases had AST level > 200 IU/L (> 5×ULN). While 9 cases (22.5 %) had AST level > 400 IU/L (> 10×ULN) in group A. **(Table 2)**

(ULN – upper limit of normal)

2 cases (5%) had AST level more than 1000 IU/L with highest enzyme level recorded was 3174 IU/L in group A.

28 (70%) cases had ALT level more than 120 IU/L (> 3×ULN), 9 (22.5 %) cases had ALT level > 200 IU/L (> 5×ULN). While only 2 cases (5 %) had ALT level > 400 IU/L (> 10×ULN) in group A. **(Table 2)**

Only 1 patient (2.5%) had ALT level more than 1000 IU/L with highest enzyme level recorded was 1079 IU/L in group A.

In this study, 5 (12.5%) cases had AST level more than 120 IU/L (> 3×ULN), 2 (5 %) cases had AST level > 200 IU/L (> 5×ULN) in group B. **(Table 3)**

Where as in the same group B, 5 (12.5%) cases had ALT level more than 120 IU/L (> 3×ULN), 3 (7.5 %) cases had ALT level > 200 IU/L (> 5×ULN). **(Table 3)**

Not a single enzyme level was recorded more than 400 IU/L (> 10×ULN) for AST as well as ALT in group B.

As AST and ALT level > 1000 IU/L were recorded only in group A and not in group B, our findings suggests that severe hepatic dysfunction in terms of rise in AST and ALT are mostly seen in patients with platelet count <1,00,000 /cmm during critical period of illness. AST or ALT >1000/L is also one of the criteria by WHO 2009 to indicate dengue disease severity.

Our findings are supportive to that of Lee L.K. who studied clinical relevance of AST and ALT for DHF and severe dengue and concluded in their study that aminotransferase levels increased with dengue severity. They also recorded maximum AST and ALT levels during febrile and critical period of dengue illness. (21)

Our findings are also consistent with Dinh the trung et al. where they studied liver involvement associated with dengue in adults of Vietnam and found that transaminase levels increased in virtually all dengue patients and correlated with other markers of disease severity where they studied coagulation profile in particular. (2)

Similarly in Brazil, Luiz José de Souza et al in their study evaluated the impact of dengue virus infection on liver function by measuring aminotransferase in blood samples from serologically diagnosed dengue patients. Aminotransferase levels were used to classify the degrees of liver damage as grade A – normal enzyme levels; grade B – increased levels of at least one of the enzymes; grade C – increased, with at least one of the enzymes being at levels higher than three times the upper reference values; grade D – acute hepatitis, with aminotransferase levels at least ten times their normal values. Of the 169 serologically confirmed cases of dengue 65.1% had abnormal aminotransferase levels and 81 cases were classified as grade B, 25 as grade C and 3 as grade D. They concluded that Liver damage is a common complication of dengue infection and aminotransferase levels are a valuable marker for monitoring these cases. (12)

Further in our study, we found negative correlation between AST (r =-0.26) and ALT (r =-0.29) with that of platelet count but that was not statistically significant (P value >0.05) in group A. **(Table 5) (Fig 1, 2)**

We found negative correlation between ALT (r =-0.28) and platelet count which was not statistically significant (P value >0.05) in group B.

For AST also we found negative correlation (r =-0.42) with that of platelet count in group B and it was statistically significant (P value > 0.05). **(Table 6) (Fig 3,4)**

When we correlated platelet count with AST and ALT in all dengue patients irrespective of platelet count, we got not only negative correlation between AST(r =-0.36) and ALT

(r =-0.39) with that of platelet count but also this correlation was statistically significant (P value <0.001). **(Table 7) (Fig 5,6)**

Our study supports the association between the rise in AST and ALT levels with increase in dengue severity which is indicated by fall in platelet count as we have found negative correlation between AST and ALT levels and platelet count in both group A and B. This suggests risk of hepatic dysfunction in terms of rise in AST and ALT increases as platelet count deteriorates in dengue.

But this negative correlation of AST and ALT with platelet count cannot be used to differentiate dengue patients with platelet count <1, 00,000 /cmm and > 1, 00000/cmm as this correlation was statistically non significant in Group A.

So dengue patients are at the risk of hepatic dysfunction irrespective of their platelet count.

Such kinds of findings are also seen in a study carried out in Bangkok where 104 patients of dengue were classified according to severity into: classic dengue, dengue hemorrhagic fever and dengue shock syndrome. Liver function tests showed that the most severely ill patients had higher levels of aminotransferases and lower levels of globulin, whereas increases in alkaline phosphatase, bilirubin and prothrombin were unrelated to the severity of the clinical status. (22)

CONCLUSION

So in conclusion, dengue provokes varying degree of hepatic dysfunction from mild increase in AST and ALT to 10 times rise in AST and ALT in severe dengue infection. So it is important to measure and monitor AST and ALT levels in dengue infected patients as elevated transaminase levels were associated with decrease in platelet count and hence with dengue severity during critical period of illness in our study. However, serum AST and ALT levels cannot be used to differentiate dengue patients with platelet count <1, 00,000 / cmm and > 1, 00000/cmm.

In dengue patients early diagnosis and proper management improves the outcome and reduce the co-morbidities. So AST and ALT measurement can be used to evaluate degree of liver damage in dengue infection as deficiency of clotting factors (synthesized by liver) can further aggravate haemorrhagic status in DHF and DSS. However, we didn't have dengue virus serotyping data.

We studied only adult dengue patient cohort and excluded children from study. Findings need to be confirmed in large study group.

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Table 1: Comparison of age in study groups

Parameters	Group A Mean ± SD (n=40)	Group B Mean ± SD (n=40)	Group C Mean ± SD (n=40)
Age (Yrs)	31.8 ± 13.7	33.57 ± 15.17	34.73 ± 9.95

Table 2: Liver Transaminase Levels and platelet count in Dengue patients with platelet Count <1, 00,000/cm (Group A)

Sr.No	Parameter	No.	Proportion (%)	Mean±SD	Min	Max	Median
1	Platelet Count < 1,00,000/cmm	40	100	52733±28220	6000	97000	48000
2	AST (IU/L)			320±513.38	23	3174	220
	Normal (5-40) IU/L	2	5				
	More than ULN ×3 (>120 IU/L)	32	80				
	More than ULN × 5(>200 IU/L)	24	60				
	More than ULN × 10 (>400 IU/L)	9	22.5				
	More than 1000 IU/L	2	5				
3	ALT (IU/L)			164±188.95	13	1079	120
	Normal (5-40) IU/L	3	7.5				
	More than ULN ×3 (>120 IU/L)	28	70				
	More than ULN × 5(>200 IU/L)	9	22.5				
	More than ULN × 10 (>400 IU/L)	2	5				

Table 3: Liver Transaminase Levelsand platelet count in Dengue patients with platelet Count >1,00,000/cm (Group B)

Sr. No	Parameter	No.	Proportion (%)	Mean±SD	Min	Max	Median
1	Platelet Count > 1,00,000 /cmm	40	100	179945±48756	110000	324000	178500
2	AST			70±80.81	17	456	38
	Normal (5-40) IU/L	24	60				
	More than ULN ×3 (>120 IU/L)	5	12.5				
	More than ULN × 5(>200 IU/L)	2	5				
	More than ULN × 10 (>400 IU/L)						
	More than 1000 IU/L						
3	ALT			55±66.46	12	341	32
	Normal (5-40) IU/L	32	80				
	More than ULN ×3 (>120 IU/L)	5	12.5				
	More than ULN × 5(>200 IU/L)	3	7.5				
	More than ULN × 10 (>400 IU/L)						

Table 4: Liver Transaminase levels and Platelet Count in control Group (Group C)

Sr. No	Parameter	No.	Proportion (%)	Mean ± SD
1	Platelet Count /cmm	40	100	282095±79121
2	AST	40	100	25.73±9.53
3	ALT	40	100	24.57±12.60

Table 5: Correlation between platelet count <100000 /cmm with ALT and AST in Group A

Correlation between	r Value	P Value
Platelet count Vs ALT	-0.26	0.11
Platelet count Vs AST	-0.29	0.067

Table 6: Correlation between platelet count>100000 with ALT and AST in Group B

Correlation between	r Value	P Value
Platelet count Vs ALT	-0.28	0.084
Platelet count Vs AST	-0.42	0.007*

* P value <0.05 (Statistically Significant)

Table 7: Correlation between platelet count with ALT and AST in dengue patients

Correlation between	r Value	P Value
Platelet count Vs ALT	-0.39	<0.0001*
Platelet count Vs AST	-0.36	0.001*

* P value <0.05 (Statistically Significant)

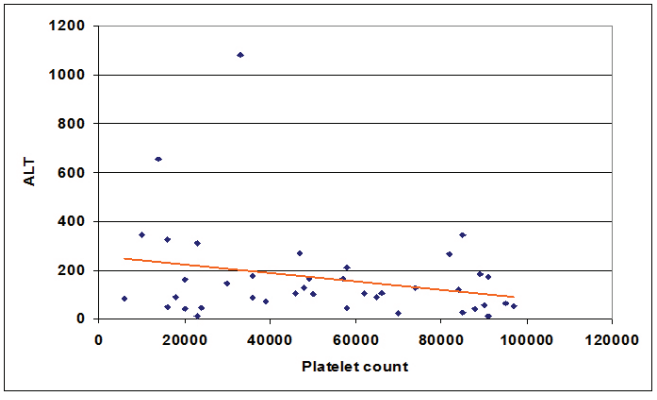

Figure 1: Scatter diagram showing correlation between platelet count<100000 and ALT in dengue patients

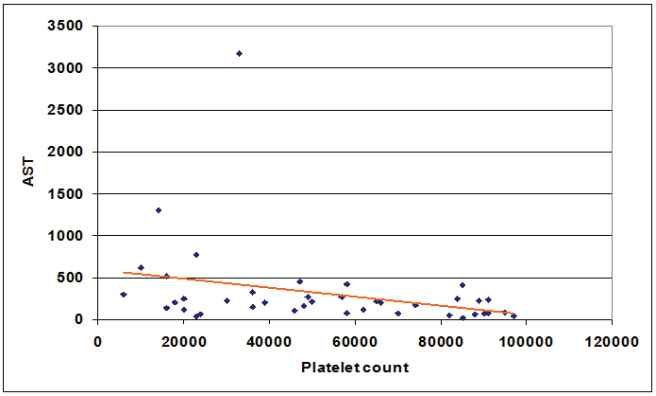

Figure 2: Scatter diagram showing correlation between platelet count<100000 and AST in dengue patients

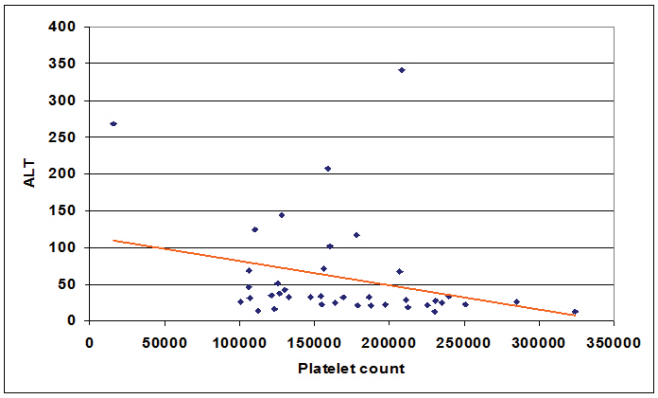

Figure 3: Scatter diagram showing correlation between platelet count>100000 and ALT in dengue patients

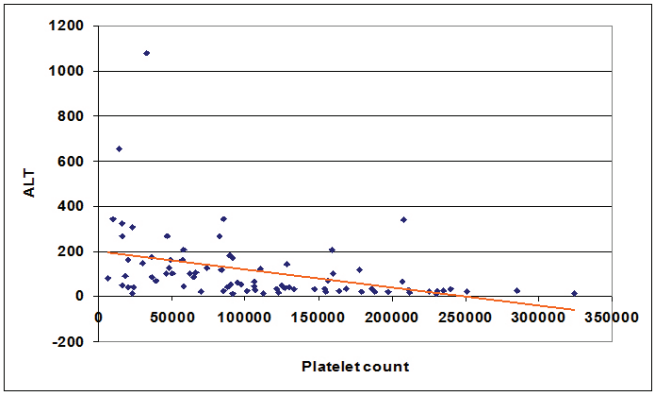

Figure 5: Scatter diagram showing correlation between platelet count and ALT in dengue patients

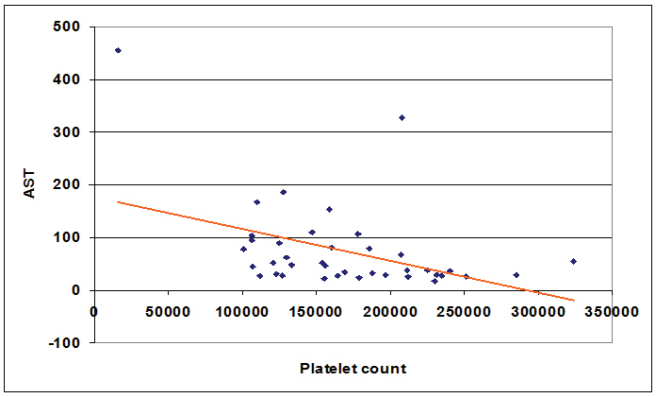

Figure 4: Scatter diagram showing correlation between platelet count>100000 and AST in dengue patients

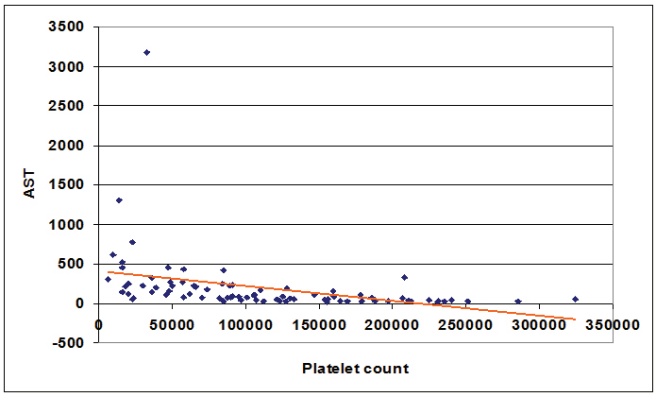

Figure 6: Scatter diagram showing correlation between platelet count and AST in dengue patients.